SKINETHIC™ HCE TIME-TO-TOXICITY : AN ALTERNATE OF THE *IN VIVO* STANDARD FOR SURFACTANTS

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1 INTRODUCTION

The SkinEthic™ HCE Time-to-Toxicity (TTT) test method was adopted by the OECD as a full replacement to the *in vivo* Draize eye test for classification of chemicals (TG 492B, 2022). This stand-alone NAM has been developed to distinguish the three UN GHS categories for liquid, semi-solid, and solid chemicals (Alépée et al., 2020, 2021, 2022).

Recently a Defined Approach has been developed by Cosmetics Europe to specifically predict the ocular hazard identification of chemicals having surfactant properties (Alépée et al., 2023; ICCS see poster 434).

Surfactants are any organic substance, intentionally added to clean, because of their surface-active properties that can lower the surface tension of liquids or interfaces of liquids. A single molecule of surfactant contains a hydrophobic group linked to a hydrophilic one. Such molecules tend to aggregate at the interfaces between the aqueous medium and the other phases of the system such as air, oily liquids, and particles, giving them properties such as foaming, emulsification, and particle suspension. Because of these properties, they are widely used in many industries such as household, pharmaceutical, agricultural, food and cosmetic sectors, leading to regulations on their use.

The purpose of the current study is intended to identify eye irritation hazard of chemicals having surfactant properties (neat and in dilution) for the purpose of classification and labelling without the use of animal testing i.e., UN GHS Cat. 1 vs. UN GHS Cat. 2 vs. UN GHS No Cat using the SkinEthic™ HCE Time-to-Toxicity.

2 MATERIALS AND METHODS

PROTOCOL and OVERALL PERFORMANCE of the TTT

LIQUIDS (TTL)

Exposure:
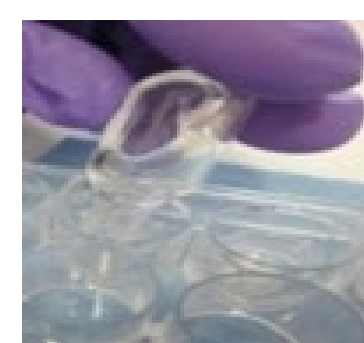5 min (Neat)
16 & 120 min (20% w/v)
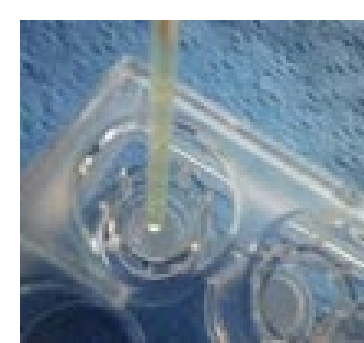
Rinse + Post Soak: 10 min

Viability assessment
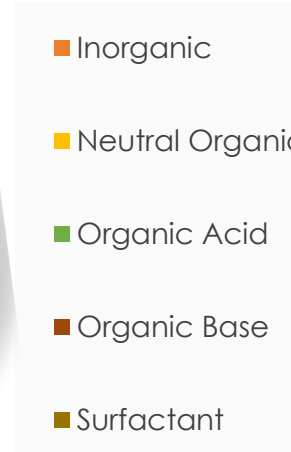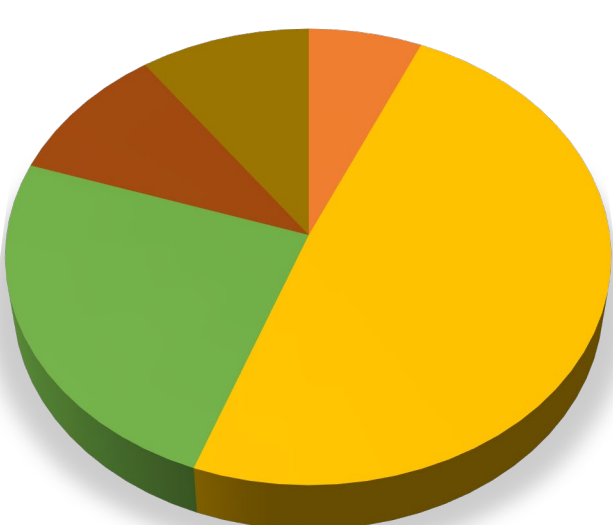
SOLIDS (TTS)

Exposure:
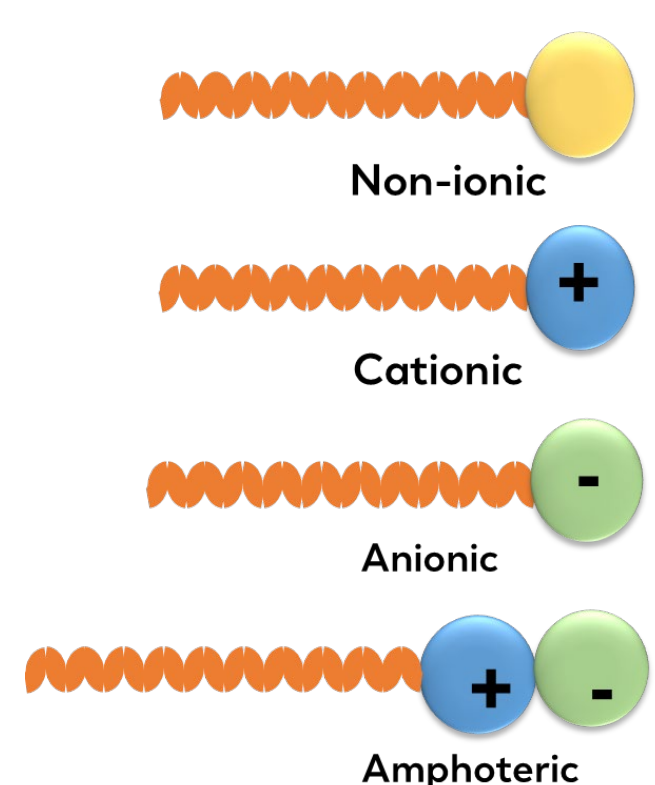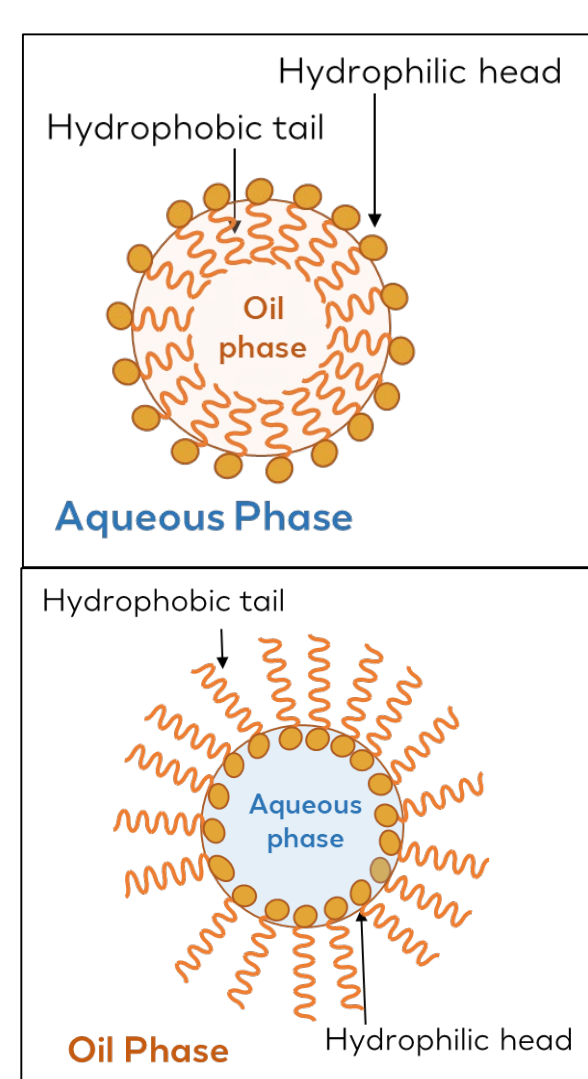30 & 120 min (Neat)

Rinse + Post Soak: 30 min

Viability assessment

All chemicals (N=151)

- Inorganic
- Neutral Organic
- Organic Acid
- Organic Base
- Surfactant

UN GHS	N	Cat. 1	Cat. 2	No Cat.
Cat. 1	50	79.2% (75%)	20.8%	0.0 %
Cat. 2	44	18.3 %	69.2 % (50%)	12.5 %
No Cat.	57	1.8%	23.4 %	74.9 % (70%)

The overall performance of the SkinEthic HCE Time-to-Toxicity to distinguish between the three UN GHS categories was compared against minimum performance values (in brackets) for each category proposed by CE and accepted by the OECD Expert Group. During the development of the NAM, 15 surfactants were considered.

SURFACTANTS EVALUATION

Hydrophilic head
Hydrophobic tail
Oil phase
Aqueous Phase

Non-ionic
Cationic +
Anionic -
Amphoteric + -

Hydrophobic tail
Aqueous phase
Oil Phase
Hydrophilic head

The validation set was further expanded to provide a more comprehensive set of 41 test items having surfactant properties. This set contained 20 UN GHS Cat. 1, 7 Cat. 2 and 14 No Cat. surfactants covering the most important drivers of classification and represented all classes (cationic, anionic, amphoteric, and non-ionic) .

The performance of the method to predict the eye hazard potential of surfactants was assessed by comparing the results with the historical *in vivo* Draize Eye classification and are summarized in Table 1 .

The performance of the SkinEthic HCE Time-to-Toxicity seemed promising, recognizing that the number of Cat. 2 surfactants was quite low compared to Cat.1 and No Cat. tested surfactants. Considering the classes of surfactants, cationic test items seemed to be the most toxic, followed by anionic, and non-ionic in decreasing order of toxicity.

3 RESULTS & DISCUSSION

Table 1: Predictions of the surfactants tested with the SkinEthic™ HCE Time-to-Toxicity

Chemical Name	CAS RN	Category	Tested Concentration	Main *in vivo* Driver	UN GHS vs TTT Cat. 1	Cat. 2	No Cat.
Cetyltrimethyl ammonium bromide	57-09-0	Cationic	10%	CO pers D21	TP#		
Benzalkonium chloride	63449-41-2	Cationic	10%	CO mean ≥ 3 CO=4	TP		
			5%	CO = 4	TP		
			1%	CO pers D21	UP		
Domiphen bromide (10%)	538-71-6	Cationic	10%	CO mean ≥ 3	TP		
			1%	No *in vivo* data		1%*	
Di(2-ethylhexyl)sodium sulphosuccinate	577-11-7	Anionic	10%	CO pers D21	TP		
Coco amidopropyl betaine	61789-40-0	Amphoteric	25%	CO pers D21	TP		
Cetylpyridinium chloride	6004-24-6	Cationic	10%	CO pers D21	TP		
			0.1%	CO = 0			TN
Sodium lauryl sulphate	151-21-3	Anionic	15%	CO pers D21	TP		
			3%	CO > 0			FP
			1%	CO > 0			FP
Ethylhexyl acid phosphate ester	12645-31-7	Anionic	Neat (Liquid)	CO mean ≥ 3	TP		
Distearyldimethylammonium chloride	107-64-2	Cationic	Neat (Solid)	CO mean ≥ 3	UP		
Ethyl lauroyl arginate HCl	60372-77-2	Cationic	Neat (Solid)	CO mean ≥ 3	TP		
Amines, C12-14 (even numbered)-alkyldimethyl, N-oxides	1643-20-5	Nonionic	28%	CO pers D21	TP		
Triton X-100	9002-93-1	Nonionic	Neat (Liquid)	IR mean > 1.5 CO = 4	TP		
			10%	Conj pers D21	TP		
			5%	CO mean ≥ 1		OP	
			1%	CO = 0			FP
1-Hexadecanaminium, N,N,N-trimethyl-, chloride	112-02-7	Cationic	25%	CO pers D21	TP		
			2%	Conj mean ≥ 2		TP	
Cetylpyridinium bromide	140-72-7	Cationic	10%	CO mean ≥ 3	UP		
			6%	IR mean > 1.5	UP		
			1%	CO mean ≥ 1		TP	
			0.1%	CO > 0			TN
Benzethonium chloride	121-54-0	Cationic	10%	CO mean ≥ 3	TP		
			1%	Conj mean ≥ 2		TP	
			0.1%	No *in vivo* data			0.1%*
N-Lauroyl sarcosine Na salt	137-16-6	Anionic	30%	CO pers D21	TP		
			10%	CO mean ≥ 1		OP	
			3%	CO > 0			FP
Deoxycholic acid Na salt (10%)	302-95-4	Anionic	10%	CO mean ≥ 1		OP	
Methyl N,N,N-trimethyl-4-[(4,7,7-trimethyl-3-oxobicyclo[2.2.1]hept-2-ylidene)methyl]anilinium sulphate	52793-97-2	Cationic	30%	CO mean ≥ 1 Conj mean ≥ 2		TP	
Polyethylene glycol monolaurate	9004-81-3	Nonionic	10%	CO = 0			TN
Polyoxyethylene 8-stearate (Myrj-45)	9004-99-3	Nonionic	10%	CO = 0			TN
Tween 80	9005-65-6	Nonionic	Neat (Liquid)	CO = 0			TN
			10%	CO = 0			TN
Polyglyceryl-3-diisooctadecanoate	63705-03-3	Nonionic	Neat (Liquid)	CO = 0			TN
Polyethylene glycol (PEG-40) hydrogenated castor oil	61788-85-0	Nonionic	Neat (Liquid)	CO = 0			TN
Cellulose,2-(2-hydroxy-3-(trimethylammonium)propoxy)ethyl ether chloride	68610-92-4	Cationic	Neat (Solid)	CO = 0			TN
Myristyl myristate	3234-85-3	Nonionic	Neat (Solid)	CO = 0			TN

majority predicted Cat.1, * No *in vivo* data, UP = Underpredicted, OP = Overpredicted, FP = False Positive, TP = True Positive, TN = True Negative

4 CONCLUSIONS

➤ 41 surfactants were evaluated using the SkinEthic™ HCE Time To Toxicity.

➤ The prediction reached the minimum performance values of 75% Cat. 1, 50% Cat. 2, and 70% No Cat. established by the OECD expert group.

➤ The OECD adopted SkinEthic HCE Time-to-Toxicity (TG 492B) can be considered in a regulatory framework for eye hazard assessment of chemicals having surfactant properties (neat and in dilution).

L'ORÉAL RESEARCH & INNOVATION

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